DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

Protozoology Applied to the Soil

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In recent years protozoology has materially widened its scope, as is evidenced by the growing interest in investigations of economic significance such as those concerning protozoa as parasites, in drinking water, etc. It needed but another step to the realization that the protozoa in the soil (and they have been observed in as large numbers as bacteria) may have a definite function in influencing soil fertility. In fact, Russell and Hutchinson[1] contend that protozoa are one of the limiting factors in soil fertility because they feed upon, and consequently limit the numbers of soil bacteria, which are for the most part, beneficial.

While this and other problems are of primary concern to the agriculturist, nevertheless their solution depends largely upon the assistance of the general protozoologist—especially in establishing certain fundamentals in methodology.

Thus at the very outset the investigator is hampered by the lack of an adequate method for counting soil protozoa. The four important methods now in use are:

(1) Rough microscopic examination, which needs no extensive criticism to point out that it is exceedingly inaccurate; (2) Dilution method[2], which is only approximate by virtue of the fact that the

[1]Russell and Hutchinson—Jour. Agr. Sci., *3* (1909): 111; *5* (1913): 152, etc.
[2]Rahn—Centr. f. Bakt. II, *36* (1913): 419.

differences between dilutions must of necessity be fairly large in rapid work; (3) Loop method[3], where the protozoa in a measured loop are counted microscopically. In the first place the amount of liquid taken up will vary with a number of samples, because of differences in surface tension—especially where various different media are employed. Secondly, the size of the platinum loop itself will vary as a result of heating and cooling (on sterilization in a flame) and chance contact with resistant surfaces; (4) The plating method[4], is necessarily limited, because some protozoa cannot grow on solid media.

It thus becomes evident that the above methods do not lend themselves to accurate work; which fact, led the authors to devise a new method for the counting of protozoa.

This method is an adaptation of the well-known blood-counting apparatus—("Blutkörperzählapparat"—Carl Zeiss-A. H. Thomas, Philadelphia). The principles underlying the use of this instrument is the microscopical observation of a drop of standard size.

The apparatus is designed as follows: A glass disc is cemented to a glass slide in the middle of a circular cavity slightly larger than the disc. A thick cover glass, the planes of which are parallel, is placed over the solution which rests on the glass disc. The excess liquid is pressed into the reservoir surrounding the disc, thus making the film of solution on the disc .1 mm. in depth. It will be seen that the difference in the depth between the glass disc and the cover glass is constant, providing the contact between the solution on the disc and the cover glass is perfect. An area one square mm. in size is marked off on the disc, and this is further subdivided into four hundred squares.

The method of procedure in the use of this apparatus is as follows: Thoroughly clean both slide and cover glass with alcohol. With a sterile platinum loop place a few drops of solution to be examined on the glass disc. It is necessary to have a film of sufficient thickness so that when the cover glass is properly adjusted and slightly pressed down, perfect contact is ensured. The preparation is then placed under the microscope and the organisms are

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[3]Müller—Arch. f. Hyg., *75* (1912): 321.
[4]Killer—Centr. f. Bakt. II, *37* (1913): 521.

examined with either low or high power. With the ordinary Blutkörperzählapparat cover glass it is impossible to use the oil-immersion, but we deem it advisable to have a special cover glass with parallel surfaces ground fine enough to permit the use of that power, because it has been observed that some very small flagellates may escape observation even when viewed with the high power lens.

The protozoa are counted in a number of fields and the average is taken. Examination of each solution was made in triplicate to ensure accuracy. It was observed that the flagellates and ciliates maintain practically the same position in the field for the brief time required for counting. The peculiar movement of each makes them easily recognizable. However, when they are counted in the living state the possibility exists of their moving from one field to another. It depends upon the number of protozoa present, and the importance of the time element, whether or not they are to be counted in the living state.

Because of their rapid motility, the large ciliates are killed before counting. This is done by passing the loopfulls of media through the vapors emanating from a bottle of osmic acid (as recommended by Goodey[5]) before placing them on the glass disc.

If it is desired to stain the organisms, this may be readily done by using equal volumes of solution and stain (e. g. water solutions of methylene blue, gentian violet, etc.) without killing the organisms. Or again the organisms may be killed and stained in one process as with picrosulphuric acid (Kleinenberg); or the same may be done in two separate steps with osmic acid vapor and other stains.

The use of low or high power lenses depends largely upon the kinds and numbers of the organisms present. It is recommended that more fields be counted with the higher than with the lower power.

The results are calculated on the basis of the area under observation to cubic volume, and then per cc. of solution used.

The advantages of using this apparatus for counting protozoa are as follows:

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[5]Goodey—Proc. Roy. Soc. Lond., *84* B (1911): 165.

(1) It is a direct method thus eliminating many errors attending incubation, etc., and the results can be reported immediately.

(2) It is more accurate than any other method in use, because it is a standard instrument and no mechanical variation is possible.

(3) It is rapid and saves considerable time in contradistinction to other methods, and the technique is simple. For example, triplicate counts on any media were recorded in ten minutes.

(4) The counts check more closely than those of the above methods used.

(5) It can be used to advantage whether the number of protozoa present be large or small.

(6) It can be used for living, killed, or stained organisms, and permits of a thorough observation of the individual organisms.

(7) The experimental error is 5%.

Its disadvantages are that the initial cost is greater than that of other methods, and the sample is too small to be representative. The error of counts is considerable where the protozoa are very few or many in number. A number of fields must be counted because of the uneven distribution, if an accurate count is required.

The starting point for a great deal of experimentation on the activity of soil protozoa is the choice of a suitable medium for the development of the organisms. Little work has been done relative to the comparison of different media, and as a general rule each investigator has a predilection for some particular one which is seldom based upon any study of the advantages and disadvantages of the media adapted to a definite line of research.

Cunningham and Löhnis[6], Killer[4], and others have presented some valuable information upon this subject, and our experiments were modeled to a great extent upon the work of the former.

In order to determine which medium would be best adapted to the large and rapid multiplication of the various kinds of protozoa, the following media were employed in dilutions of .5%, 1%, 3% 5%, and 10%. Dried blood, hay infusion, hay infusion plus .5% egg albumen (Goodey)[5], peptone, horse, cow, and chicken manures (Mar-

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[6]Cunningham and Löhnis—Centr. f. Bakt., II, *39* (1914): 596; ibid. 42 (1914): 8.
[4]Killer—loc. cit.
[5]Goodey—loc. cit.
[6]Löhnis—loc. cit.

tin)[7], egg albumen, bouillon, and soil extract (Löhnis)[6] in dilutions of 400 cc., 600 cc., 800 cc., 1000 cc., 1200 cc., per kg. of soil.

The results obtained in this survey may be condensed in the following table which indicates the maximum numbers of protozoa appearing on the most favorable medium every day, for five successive days, as determined by the new counting method previously described. It had been previously determined that the count did not increase materially after the fifth day, consequently there was no need of continuing the work for any longer period of time.

Since the object was to determine total numbers of protozoa in the soil, rather than species, no differential count is appended.

Maximum Numbers and Media

Days	Large Ciliates	Large Flagellates	Small Ciliates	Small Flagellates
1	8,520 in Soil Ex. 800 cc.	840 in 10% Hay	4,255 in 5% D. B.	28,750 in 5% D. B.
2	63,800 in Horse 5%	709 in 5% Egg Albumen	9,210 in 3% Chicken	282,000 in .5% Horse
3	319,010 in Hay 10%	10,625 in 10% Hay	208,000 in 3% Chicken	636,500 in Soil Ex. 1000 cc.
4	708,000 in Hay 10%	7,435 in 5% Cow	379,000 in 3% Egg	478,000 in 1% Horse
5	1,410,000 in 10% Hay & Egg	31,900 in 5% Cow	804,000 in 3% Egg	1,878,000 in 3% Hay & Egg

Summarizing the work outlined above, it was found:

(1) The new method for counting protozoa consists of an adaptation of the Blutkörperzählapparat whereby the organisms may be counted directly, rapidly and accurately. It has been used successfully in the comparison of media for the development of protozoa and other experiments.*

(2) 10% hay infusion proved to be the most favorable medium for the development of large numbers of small flagellates, as well as small and large ciliates. Hay infusion in various concentrations with, and without, the addition of egg albumen, proved to be well adapted to the development of the organisms. Hay infusion plus 5% egg albumen proved superior to all other media for the development of ciliates.

(3) Soil Extract is an excellent medium, though somewhat inferior to hay infusion plus 5% egg albumen, and with the soil used in this experi-

[7]Martin and Lewin—Phil. Trans. Roy. Soc. Lond., B. *205*: 74.

ment lower concentrations than those recommended by Löhnis developed protozoa in a shorter period of time.

(4) 3% chicken manure is an excellent medium for the development of small ciliates.

(5) The numbers and species of protozoa which can be obtained from a given soil are largely dependent upon the media employed, time of incubation, as well as the kind of soil used.

(6) In general the order of appearance of protozoa was as follows: small flagellates, small ciliates, large flagellates and finally large ciliates. This is in accordance with Cunningham and Löhnis' observations.

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*Note: Further results on experimentation and a complete bibliography on soil protozoa and soil sterilization are awaiting publication.

The Presence of Acidophilous Cells in the Adrenals of Certain Amphibians*

By Thomas Byrd Magath

The *adrenal glands* of the *Amphibians* vary, in color, from a light yellow to a golden reddish-yellow, and extend along the ventral side of the kidneys in a thin band. The function of these ductless glands is not definitely known, altho it is known that an injection of "adrenalin" will considerably increase the blood pressure and that the removal of both glands will result in death, as does an overdose of the extract.

Stilling found in the study of the adrenals of the Rabbit, during the summer, some remarkable cells which stained an intense red with eosin and appeared very granular in structure. He also found cells in *Rana esculenta* which had the same appearance and stained like those in the Rabbit; because of their appearance in the summer he called them *"Summer cells"*.

Patzelt and Kubik, however, found these cells all the year round and because of their affinity for acid stains called them *"acidophilous cells"*. The result of their work is as follows:

(a) There is present in the adrenal glands of *Rana esculenta*, as in the case of Mammals, Birds and Reptiles, two kinds of cells, viz., an epithelial portion and a *chromaffine* portion. The chromaffine portion is made up of large granular cells which turn a brownish-yellow when treated with chromic acid or its salts.

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*Contributions from the Biological Laboratory, James Millikin University, No. 12.